

Influence of land use on metal concentrations in playa sediments and amphibians in the Southern High Plains

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Land use surrounding playas has little effect on metal concentrations in sediments.

Abstract

The Southern High Plains (SHP) is a semi-arid region in which playa wetlands are the focal points of biodiversity. Playas are highly influenced by surrounding land use. Most of the SHP is in agricultural production (primarily cotton) with a history of arsenic-containing herbicide use. Metals influence reproduction and development in amphibians. We analyzed metal residues in playa sediment and whole body tissue of *Spea* spp. and *Bufo cognatus* metamorphs from two land uses: cropland and native grassland. Cd and Ni concentrations in *B. cognatus* tissues differed between land uses. Metal concentrations in *Spea* spp. tissues did not differ between land uses. Ba was higher in *Spea* spp. than *B. cognatus* collected from the same grassland playas, indicating differential habitat use. No correlations between sediment and tissue concentrations were found. Land use appeared to have little influence on metal concentrations and levels were below those known to cause effects in amphibians. © 2006 Elsevier Ltd. All rights reserved.

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1. Introduction

Amphibians are sensitive to environmental and anthropogenic influences (Kiesecker et al., 2001) and often used as indicator species of ecosystem health (Collins and Storer, 2003). Amphibians can accumulate metals, often inducing toxic effects in adults and larvae (reviewed by Hall and Mulhern, 1984). Aquatic and terrestrial forms of amphibians are good indicators of metal contamination because of their close contact with sediment and soil, either through direct contact (e.g., burrowing) or diet (Hall and Mulhern, 1984; James et al., 2004). Larval amphibians may accumulate metals more readily than adults, possibly due to differences in surface

area to volume ratios and skin permeability (Hall and Mulhern, 1984; Judd, 1977); however, examples of older stages being more sensitive exist (Freda, 1991). Metals can adversely affect multiple aspects of amphibian physiology, biochemistry, and behavior, including reduced survival, stunted growth, and lowered fright response (Arrieta et al., 2004; Bridges, 1999; Lefcort et al., 1998; Loumbourdis et al., 1999). In addition, younger stages of amphibians can be more susceptible to contaminants than older stages, possibly due to differential tolerance or sensitivity between age groups (Freda, 1991; Judd, 1977; Pérez-Coll and Herkovits, 1996). Moreover, accumulation of metals and their toxicity in amphibians can be influenced by complex interactions between individual metals, and metal interactions with water quality parameters such as pH, DOC, and water hardness (Freda, 1991; Herkovits and Pérez-Coll, 1991; Horne and Dunson, 1995a,b; Sparling and Lowe, 1996).

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Playas are shallow depressional wetlands that are most numerous in the Southern High Plains (SHP) of northwest Texas and eastern New Mexico (Smith, 2003). The estimated 20,000 playa wetlands in the SHP, average 6.3 ha, occupy approximately 2% of the area (Guthery and Bryant, 1982), and are the focus of biodiversity in this landscape (Bolen et al., 1989). Thirteen species of amphibians inhabit playas in the SHP (Anderson et al., 1999; Bolen et al., 1989; Haukoos and Smith, 1994).

Farming is the dominant land use on the SHP, and pesticides are intensively used to control plant and invertebrate pests (Thurman et al., 2000). Cotton production constitutes most of the agricultural activity in the region, although other crops such as sorghum, wheat, and sunflowers are grown (Smith, 2003). Arsenic-based and organochlorine pesticides persist in the environment and were widely used in the SHP (Guerrant et al., 1970). In 2000, farmers used a total of 60,000 lbs (27215.5 kg) of monosodium methanearsonate (MSMA) active ingredient on upland cotton in Texas (U.S. Department of Agriculture, 2005), most of which is grown in northwest Texas. Although playa basins are not typically planted in cotton, agricultural activities in surrounding watersheds promote significant sediment runoff into playas (Luo et al., 1997). Increased sedimentation in playas potentially concentrates contaminants derived from natural and anthropogenic sources (Irwin et al., 1996). Based on particle size analysis, Luo et al. (1999) determined that sediments in playas are predominantly of waterborne deposition.

Playa volume has decreased due to sedimentation (Luo et al., 1997) thereby reducing hydroperiod. Recent playa amphibian studies (Ghioca, 2005; Gray et al., 2004a,b; Gray and Smith, 2005; Smith et al., 2004) have shown correlations between shortened hydroperiod and alterations in amphibian metamorphosis time and decreased postmetamorphic body size (Gray and Smith, 2005). Strong correlations exist between shortened hydroperiods and alterations in amphibian development (Denver et al., 1998; Newman, 1989), yet contaminant exposure also may influence amphibian development. Despite the extent of agricultural activity in this region and playa wetlands being the central repository for runoff from surrounding fields (Luo et al., 1999), little is known about contaminants in playas, and no data have been collected on contaminants in playa amphibians.

Due to past use of metal-based pesticides in the region and the potential for concentration of naturally occurring metals in sediments of playas, we hypothesized that sediment from playas with cropland watersheds (i.e., cropland playas) would have higher concentrations of metals, especially As, than playas in native grassland watersheds (i.e., grassland playas). In addition, we expected tadpoles to reflect concentrations of metals found in playa sediments, and recently metamorphosed individuals to represent the entire larval exposure history. Thus, we hypothesized that metal concentrations would be greater in recently metamorphosed amphibians collected from cropland playas than grassland playas, and that variation in metal concentrations in amphibians would correlate with variation of metals in playa sediments, regardless of land use type.

2. Methods

Playas were categorized as either cropland ($n = 6$) or grassland ($n = 6$) if more than 75% of their respective watersheds were either in row crop agriculture or native grassland, respectively. One sediment sample was collected on 23 July 2003 from each of 12 playas located within 22 km of each other in Floyd County, TX, USA (N 33°56', W 101°10'). No playa was within more than 0.8 km of any other playa sampled. Sediment also was collected from three additional grassland playas in Deaf Smith County, TX, USA (N 34°15', W 102°50'). Sediment from two playas in Deaf Smith County were sampled on 26 August 2003 and sediment from the third playa on 16 September 2003. Playas in Deaf Smith County were in a grassland-dominated area (across multiple watersheds) and thus would not be considered "grassland islands" like many grassland playas in Floyd County. Deaf Smith playas were separated from the other grassland playas for this reason and treated separately throughout the analyses. Organic carbon content is 1.2–1.7% in the top 15 cm of playas and decreases with depth (Luo, 1994). Therefore, sediment was scooped from the top strata of sediment (10 cm) within each playa using 500 ml chemically clean glass jars.

We studied the dominant anurans, Great Plains toad (*Bufo cognatus*) and spadefoot toads (*Spea* spp.) in playas. Metamorphosed *Spea* spp. recently emerged from two cropland and five grassland playas and *B. cognatus* from four cropland and four grassland playas were collected concurrent with sediment samples. *S. multiplicata* and *S. bombifrons* are included in the *Spea* spp. complex as they are difficult to distinguish at this age. *B. cognatus* recently emerged from the Deaf Smith County playa sampled in September were collected concurrent with sediment. This sample was not included in statistical analyses to avoid confounding results due to potential location and age differences. Amphibians were collected from the edge of the playa, euthanized using tricaine methane sulfonate (MS-222), and stored in Whirlpak bags on ice for transport back to the laboratory.

All sediment and tissue samples were stored at -20°C until processed. Sediment and tissue samples were processed within 6 months of collection. Two to three samples were analyzed per playa consisting of one sediment sample and either one *B. cognatus* sample or one *Spea* spp. sample, or samples from both species, if collected. A total of 134 *Spea* spp. and 95 *B. cognatus* were collected from 13 playas. Four to 39 *Spea* spp. metamorphs (3.28–27.20 g, composite wet wt.) and 2–16 *B. cognatus* metamorphs (6.66–22.78 g, composite wet wt.) were pooled from each playa for residue analysis because individuals were too small as individuals for instrument detection limits.

Sediment samples were dried at room temperature to a constant mass and homogenized by hand grinding with a mortar and pestle. Extraneous organic matter (e.g. grass, roots) was removed during grinding and sifting (mesh size = 2 mm). Triplicate samples of sediment from the same sampling jar for each playa were weighed (0.50 g) and digested using EPA method 3050B (Environmental Protection Agency (EPA), 1996). Two replicates of standard reference material (SRM) Montana soil 2710 from National Institute of Standards and Technology (NIST; Gaithersburg, MD, USA), and four washes of collection sieves were also included in the digestion process. Ten milligrams of 50% HNO_3 was added to each sample. Samples were refluxed on hot plates while adding 5 ml aliquots of concentrated HNO_3 every 30 min (5–7 times) until digestion was complete. Samples were evaporated to 5–10 ml and cooled. Two ml of MilliQ water (distilled, deionized water with resistivity $>18\text{ M}\Omega$) and hydrogen peroxide (30%, 3 ml) were added and refluxed. Seven 1-ml aliquots of hydrogen peroxide were added until effervescence diminished. Samples were evaporated to 5 ml, cooled, and concentrated HCl (5 ml) added. Samples were refluxed for 20–30 min and then cooled. Samples were then filtered with Whatman No. 1 (Whatman International Ltd., Maidstone, UK) filter paper and diluted to 25 ml.

Samples were analyzed for Al, As, Ba, Be, Cd, Cr, Cu, Ni, Pb, and Zn using Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) (Leeman Labs Inc. Direct Reading Echelle) with a calibration curve of five standards ranging from 0.050 to 10 ppm. The lowest point on the calibration curve was at least twice the calculated instrument detection limit (IDL) for each element. Hg was not analyzed in sediment samples because the allowable holding time for samples was exceeded for Hg analysis.

Two to 11 *Spea* spp. and *B. cognatus* individuals from each playa were measured to determine body mass and snout-vent length (SVL), and a portion of those (2–5 individuals, composited) were dried at 90°C to a constant mass.

Remaining *Spea* spp. and *B. cognatus* metamorphs were composited within each playa, weighed, and digested following a modified EPA 3050B protocol. Two replicates of 2 SRMs (DORM-2 and DOLT-2, ~0.25 g) from National Research Council of Canada (NRC; Ontario, Canada) and an acid blank for both species were included. Samples were predigested overnight with approximately 50 ml (toads) or 10 ml (SRMs and blanks) concentrated HNO₃. Samples were refluxed on hot plates and 3 aliquots of concentrated HNO₃ (5 ml) added. Samples were evaporated to 15–20 ml and cooled. MilliQ water (2 ml) was added and then hydrogen peroxide added in aliquots (30%, three 1-ml aliquots for *Spea* spp., four 1-ml aliquots for *B. cognatus*) while heating until effervescence diminished upon further addition of hydrogen peroxide. Samples were cooled and then filtered with Whatman No. 1 filter paper. The DORM-2 SRMs were spiked with 50 µl of 100 ppm Pb standard to produce quantifiable lead concentrations in that SRM. Tissue samples were diluted to 50 ml while SRMs and blanks were diluted to 25 ml.

Tissue samples were separated into two aliquots to be analyzed on ICP-AES for the same metals as the sediment and for Hg using cold vapor AA (Perkin Elmer FIMS 100). Tissues were analyzed twice for two different groups of metals based on experience from analyzing sediment samples. Al, Ba, Cu, and Zn was analyzed using five standards ranging from 0.050 to 10 ppm while As, Be, Cd, Cr, Ni, and Pb was analyzed on a range of five standards from 0.010 to 2 ppm. Tissue was analyzed for Hg by cold vapor AA using six calibration standards ranging from 1 to 32 ppb.

2.1. Statistical analyses

Metal concentrations were calculated after averaging triplicates for each playa sediment sample. One cropland and one grassland playa only had duplicates. All metal concentrations in sediment were within the range of the calibration standards. Most tissues contained metal concentrations within range of the calibration standards; however, those falling below the standard curve were dealt with in two ways. Concentrations between the lowest calibration standard and half the lowest calibration standard were above the IDL, and were reported as the values determined by the instrument. Concentrations falling below one-half the lowest calibration standard were considered non detects and were assigned values of one-half the IDL (i.e. one-fourth of the lowest calibration standard). Concentrations of Cd, As, Ni, and Hg in tissues from grassland playas were the only values replaced using this technique.

Statistical analyses were conducted using R (R Development Core Team, 2004). All variables were tested for normality. Concentration of metals in sediments and SVL of *B. cognatus* were tested for differences between land uses using a one-way ANOVA. Wet weight of both species, SVL of *Spea* spp., concentration of Cu in *Spea* spp. tissue, and concentration of Hg in tissues were tested for land use differences using a Kruskal–Wallis sum rank test. Metal concentrations in tissue were compared between species using a one-way ANOVA in grassland playas where both species were collected. Metal concentrations in tissues of each species were analyzed as the response variable and metal concentration in sediment collected from playas and land use as the independent variables in an analysis of covariance. Tissues were analyzed separately due to small sample size and uneven distribution of species among playas. The relationship between concentrations of metals in tissue and sediment were assessed for Al, As, Ba, Cd, Cr, Cu, Ni, Pb, and Zn. Tissue concentrations were designated as the response factor with sediment concentrations and land use the covariate and independent variable, respectively. Significance was set at $p \leq 0.05$.

3. Results

Body weight (wet) and SVL for *B. cognatus* were similar between land uses (Table 1). *Spea* spp. collected from cropland playas were larger (SVL; $p = 0.005$) and about 27% heavier ($p < 0.001$) than those from grassland playas (Table 1).

No metal residues were found in washes of collection sieves. Mean percent recovery was determined for each analyte (Table 2). Even though these recoveries were variable, all analyzed metals were detected in all sediment samples from cropland and grassland playas (Table 3). Most metal concentrations in sediments did not differ between cropland and either category of grassland playa. The only exception was Ba, for which the concentration from sediments in the grassland-dominated landscape (Deaf Smith County) was 53% greater than sediment in grassland or cropland playas in Floyd County ($p = 0.008$; Table 3).

Most metals, with the exception of Be, were detected in *Spea* spp. and *B. cognatus* composites (Table 3). Beryllium was below the detection limit for all tissue samples. Concentrations of all metals were similar between land use types for *Spea* spp. (Table 3). Concentrations of Cd and Ni in *B. cognatus* metamorphs differed between land uses (Cd: $p = 0.047$, Ni: $p = 0.029$; Table 4). Mean concentrations of Ni were two-fold greater in *B. cognatus* collected from cropland than grassland playas. Conversely, concentrations of Cd were higher in toads from grassland playas, although mean differences were small (Table 3). No linear relationships existed between whole body tissue and sediment concentrations (regardless of land use) for either species of toad (Tables 4 and 5). Only one tissue sample was analyzed from Deaf Smith County and therefore was not included in this analysis. Metal concentrations in that sample were generally similar (Ba, Cd, Cr, Cu) or much lower (Al, As, Hg, Ni, Pb; except Zn which was slightly higher 11.7 µg/g) than *B. cognatus* tissues from Floyd County playas. In grassland playas where both species were collected, *Spea* spp. accumulated more than 4 times as much Ba as *B. cognatus* ($p < 0.001$; Table 6).

4. Discussion

We hypothesized that metal concentrations in playa sediments would differ between cropland and grassland playas. Agricultural use of pesticides containing As could result in more of this contaminant in cropland playas, and runoff of

Table 1
Snout-vent length (SVL; cm) and wet and dry weights (g) for amphibians collected in cropland and grassland playas in the Southern High Plains in 2003

Species	Land use	Wet weight ^a	Dry weight ^b	SVL ^{a,c}
<i>Spea</i> spp.	Grassland	0.89 ± 0.11 ($n = 35$)*	0.51 ± 0.20 ($n = 4$)	2.1 ± 0.4 ($n = 35$)*
<i>Spea</i> spp.	Cropland	1.13 ± 0.06 ($n = 17$)*	0.64 ± 0.22 ($n = 2$)	2.4 ± 0.2 ($n = 17$)*
<i>B. cognatus</i>	Grassland	1.69 ± 0.16 ($n = 33$)	0.37 ± 0.07 ($n = 2$)	2.7 ± 0.5 ($n = 33$)
<i>B. cognatus</i>	Cropland	1.67 ± 0.24 ($n = 23$)	0.47 ($n = 1$)	2.7 ± 0.6 ($n = 23$)

*Denotes significant difference between cropland and grassland playas for *Spea* spp.

^a Sample size (n) is the number of individuals measured for each species and treatment.

^b Sample size (n) is the number of composites per playa for each species and treatment.

^c SVL (snout-vent length) is a sub-sample of amphibians digested and all of those dried.

Table 2
Percent recovery for each metal after digestion of sediment and whole body tissue

SRM	Al	As	Ba	Be	Cd	Cr	Cu	Hg	Ni	Pb	Zn
MT soil 2710	39.1	78.6	38.5	NA ^a	57.3	42.1	87.7	— ^b	59.1	85.1	77.4
DOLT-2	81.3	63.8	NA	NA	82.9	BDL ^c	87.9	47.2	BDL	88.4 ^d	89.5
DORM-2	97.3	80.1	NA	NA	BDL	38.5	BDL	32.8	32.3	BDL	95.2

Standard reference material used for sediment was Montana Soil 2710 from NIST and for tissue was DOLT-2 and DORM-2 from NRC.

^a NA, reference values are not available.

^b Soil samples were not analyzed for mercury.

^c BDL, Certified amount was below our method detection limit.

^d Calculated from ~0.28 g SRM + Pb spike because of low Pb concentration in DOLT-2.

sediment into playas in cultivated watersheds could concentrate metals existing naturally in surface soils. Inundation and windy conditions mixes upper sediments to such an extent that playa sediments cannot be aged (Smith, 2003: 20). We feel that this continuous mixing homogenizes sediments and possible contaminants, thereby allowing one sediment sample to be representative of concentrations of contaminants found throughout the playa. Nonetheless, we failed to observe any differences in metal concentrations between cropland and grassland playas, with the exception of Ba, which demonstrated greater concentrations in grassland playas in Deaf Smith County compared to cropland and grassland playas in Floyd County. The significance of this difference is unclear, although the playas we sampled in Deaf Smith County are embedded in a more contiguous grassland landscape than those in Floyd County, and are located 180 km northwest of Floyd County sites.

To our knowledge, only one study exists on metal concentrations in sediments in playa wetlands (Irwin et al., 1996). Similar to our results, Irwin et al. (1996) found that concentrations of most metals were similar between grassland playas and those in cultivated watersheds. In addition, concentrations of metals in our sediment samples were similar to those observed by Irwin et al. (1996). Irwin et al. (1996) expressed

concern about As levels in sediment which were above background levels defined by the International Joint Commission (IJC in Irwin et al., 1996). Arsenic levels are typically considered elevated in much of the SHP due to its use in pesticide formulations. Background levels of As in soil average about 5 mg/kg (Agency for Toxic Substances and Disease Registry (ATSDR), 2000). Thus, concentrations of As in playa sediments in this study were generally 6 to 7 times greater than worldwide soil background levels.

Amphibians accumulate metals from the environment, and overall, larval amphibians likely accumulate metals at a greater rate than adults (Hall and Mulhern, 1984). Differences in integument, diet, and simply the media in which the organism lives, influence the potential for accumulating metals. We predicted that metal concentrations in amphibians would differ between grassland and cropland playas. Metals accumulated in tissues of amphibians, with all metals (except Be) detected in whole body tissues of *Spea* spp. and *B. cognatus*. Beryllium is generally associated with mining or aerospace activities which does not occur in this region so the lack of Be is not surprising (Agency for Toxic Substances and Disease Registry (ATSDR), 2002). In all cases, whole body tissue concentrations of *Spea* spp. and *B. cognatus* were substantially lower than concentrations found in sediment. Thus, although metals

Table 3
Mean \pm SE (μ g/g) metal concentrations found in sediment and whole body tissue of *Spea* spp. and *Bufo cognatus* metamorphs collected from two different playa types in the Southern High Plains in 2003

Metal	Sediment ^a			<i>Spea</i> spp. ^a		<i>B. cognatus</i> ^a	
	Cropland	Grassland (F) ^b	Grassland (DS) ^b	Cropland	Grassland	Cropland	Grassland
Al	43 800 \pm 5300	35 800 \pm 3480	48 000 \pm 8040	125 \pm 22	201 \pm 56	255 \pm 40	185 \pm 48
As	35.4 \pm 3.89	29.8 \pm 2.85	38.5 \pm 5.90	0.11 \pm 0.03	0.18 \pm 0.05	0.33 \pm 0.09	0.12 \pm 0.05
Ba	124 \pm 13.8	124 \pm 10.7 ^c	190 \pm 14.7 ^c	18.1 \pm 5.8	21.7 \pm 1.3	3.9 \pm 0.4	4.8 \pm 0.4
Be	1.18 \pm 0.13	1.08 \pm 0.09	1.28 \pm 0.17	<0.02 ^e	<0.02 ^e	<0.01 ^e	<0.03 ^e
Cd	1.10 \pm 0.12	1.02 \pm 0.08	1.25 \pm 0.16	0.02 \pm 0.007	0.03 \pm 0.004	0.03 \pm 0.002*	0.04 \pm 0.003*
Cr	23.5 \pm 2.34	21.1 \pm 1.62	25.4 \pm 2.85	0.14 \pm 0.002	0.19 \pm 0.018	0.22 \pm 0.024	0.17 \pm 0.022
Cu	15.8 \pm 1.79	14.9 \pm 1.32	18.9 \pm 2.19	1.33 \pm 0.30	1.64 \pm 0.12	1.46 \pm 0.21	1.51 \pm 0.15
Hg	NA ^d	NA	NA	0.018 \pm 0.005	0.020 \pm 0.009	0.016 \pm 0.006	0.003 \pm 0.001
Ni	12.5 \pm 1.21	11.1 \pm 0.99	13.7 \pm 1.47	0.23 \pm 0.13	0.11 \pm 0.03	0.17 \pm 0.02*	0.08 \pm 0.02*
Pb	22.6 \pm 2.45	21.8 \pm 1.54	24.7 \pm 2.88	0.36 \pm 0.04	0.49 \pm 0.04	0.37 \pm 0.03	0.40 \pm 0.06
Zn	55.0 \pm 7.24	53.1 \pm 4.46	65.4 \pm 6.92	7.90 \pm 0.40	8.46 \pm 0.57	10.8 \pm 1.85	10.2 \pm 0.60

*Denotes a significant difference between cropland and grassland playas for that species and metal.

^a Sediment: cropland $n = 6$, grassland $n = 6$; Tissue composites: *Spea* spp. cropland $n = 2$, *Spea* spp. grassland $n = 5$, *B. cognatus* cropland $n = 4$, *B. cognatus* grassland $n = 5$.

^b Grassland playas from Floyd County (F) and Deaf Smith County (DS; $n = 3$) (see Section 2 for complete description of collection sites).

^c Deaf Smith grassland different from other grassland and cropland sediments ($p = 0.008$).

^d NA, sediment samples were not analyzed for Hg.

^e Computation based on mean sample mass within each species and land use type and one-half the lowest calibration standard.

Table 4

p-Values from analysis of covariance of metal concentrations in whole body tissue of *Bufo cognatus* metamorphs collected from the Southern High Plains in 2003

Metal	Sediment ^a	Land use ^b	Sediment: Land use
Al	0.254	0.267	0.469
As	0.438	0.070	0.260
Ba	0.114	0.065	0.521
Cd	0.361	0.047*	0.082
Cr	0.334	0.259	0.422
Cu	0.282	0.633	0.256
Hg	NA	0.054	NA
Ni	0.913	0.029*	0.148
Pb	0.410	0.565	0.325
Zn	0.353	0.992	0.676

*Denotes a significant *p* value ($p < 0.05$), indicating that land use is the influencing factor while accounting for affects from sediment.

^a Cropland playas $n = 4$, grassland playas $n = 6$.

^b Land use $n = 2$ (cropland vs. grassland).

accumulated, they did not show any tendency to concentrate in either species.

Although *Spea* spp. and *B. cognatus* accumulated metals, results suggest differential patterns of accumulation dictated by land use. For example, Cd and Ni concentrations in tissue were higher and lower, respectively, in *B. cognatus* collected from grassland than cropland playas. In addition, As and Hg concentrations were 3- to 5-fold greater, and Ba was 19% lower, in *B. cognatus* collected from cropland playas. Metal concentrations in *Spea* spp. tissue did not differ between land use types, although this result must be cautiously interpreted because *Spea* spp. were analyzed from only 2 of 6 cropland playas. Samples of both species were collected at four grassland playas. In general, no differences of metal concentrations in tissue were seen between species, with the exception of Ba (Table 6). Accumulation of any contaminant into an organism is dictated by a variety of factors, including diet, habitat use, duration of exposure, and individual physiological traits (Freda, 1991; Judd, 1977; Sparling and Lowe,

Table 5

p-Values from analysis of covariance of metal concentrations in whole body tissue of *Spea* spp. metamorphs collected from the Southern High Plains in 2003

Metal	Sediment ^a	Land use ^b	Sediment: Land use
Al	0.548	0.984	0.739
As	0.923	0.632	0.750
Ba	0.593	0.676	0.105
Cd	0.828	0.328	0.342
Cr	0.708	0.491	0.883
Cu	0.407	0.198	0.115
Hg	NA	0.909	NA
Ni	0.979	0.377	0.082
Pb	0.492	0.129	0.761
Zn	0.225	0.225	0.341

*Denotes a significant *p* value ($p < 0.05$), indicating that land use is the influencing factor while accounting for affects from sediment.

^a Cropland playas $n = 2$, grassland playas $n = 5$.

^b Land use $n = 2$ (cropland vs. grassland).

Table 6

Mean \pm SE ($\mu\text{g/g}$) metal concentrations found in whole body tissue of *Bufo cognatus* and *Spea* spp. metamorphs collected from the same grassland playas in the Southern High Plains in 2003

Metal	<i>B. cognatus</i> ^a	<i>Spea</i> spp. ^a
Al	212 \pm 51	204 \pm 73
As	0.14 \pm 0.05	0.17 \pm 0.06
Ba	5.0 \pm 0.5*	22.5 \pm 1.4*
Cd	0.04 \pm 0.003	0.04 \pm 0.002
Cr	0.18 \pm 0.024	0.19 \pm 0.022
Cu	1.55 \pm 0.19	1.75 \pm 0.03
Hg	0.004 \pm 0.001	0.022 \pm 0.012
Ni	0.08 \pm 0.02	0.10 \pm 0.04
Pb	0.43 \pm 0.06	0.52 \pm 0.03
Zn	10.3 \pm 0.76	8.9 \pm 0.53

*Ba concentrations differed between species ($p < 0.001$).

^a For both species, $n = 4$.

1996). In addition, bioavailability of individual metals plays a pivotal role in their uptake by any organism (Linder and Grillitsch, 2000; National Research Council, 2003).

Spea spp. and *B. cognatus* differ in a number of life history traits that could play a role in their specific exposure scenario, including the length of development period, size, and potentially diet. *Spea* spp. have a very short larval period (14–21 days; Degenhardt et al., 1996; King, 1960) while *B. cognatus* often has a longer aquatic exposure (18–49 days; Krupa, 1994). Also, *Spea* spp. tadpoles are larger than *B. cognatus* tadpoles throughout the larval period, resulting in a greater surface area to volume ratio for *B. cognatus*. Finally, *Spea* spp., particularly *S. bombifrons*, may transform into carnivorous morphs, completely shifting diet during the larval period (Pfennig, 1992). *B. cognatus* graze submerged surfaces for organic and inorganic matter while *Spea* spp. eat invertebrates, algae, and detritus (Farrar and Hey, 2005; Graves and Krupa, 2005; Morey, 2005).

Metal concentrations in sediment were at least 5 times higher than concentrations in tissue, which indicates that bioaccumulation of metals did not occur. However, the route of exposure (e.g., prey items, accidental ingestion of soil) is important in determining the actual concentrations found in amphibians (Linder and Grillitsch, 2000). No relationships between metal concentrations in sediment and tissue were found in our study. Bioavailability of metals in sediment and food items depends on the chemical processes in the environment, the trophic level position of the receptor organism, the metal, and the metal ion species (Chen et al., 2000; Linder and Grillitsch, 2000; National Research Council, 2003).

Concentrations of most metals in this study were below concentrations detected in amphibians in other residue studies and much lower than concentrations causing effects in other species. Concentrations of Cr, As, and Zn in sediment at a site known to be contaminated were much higher than concentrations of metals in this study (Clark et al., 1998). Similarly, tissue concentrations in this study were much lower than concentrations of metals determined in tadpoles by Clark et al. (1998). Concentrations of Pb in species from this study were much less than concentrations in *Xenopus laevis* exposed to soil with Pb concentrations similar to those found in our

study and water (Berzins and Bundy, 2002). Concentrations above environmentally relevant levels caused delayed development and reduced body weight (Berzins and Bundy, 2002). Wet weight concentrations of all metals except As and Cd in tissues in our study were much lower than dry weight concentrations in *Rana clamitans* (Gilliland et al., 2001). Differences are minimal and attributed to difference of wet versus dry weight and between species. More importantly, Gilliland et al. (2001) found very few deformities among the frogs they sampled, suggesting that the concentrations determined do not cause visible defects in that species. The 96-h LC₅₀ for *Rana ridibunda* tadpoles was determined as 71.8 ppm Cd (Loumbourdis et al., 1999). The tadpole group exposed to 12.5 ppm Cd over 30 days had 70% mortality, the survivors a body burden of >25 ppm Cd, and weighed less than tadpoles in the control group. Metal concentrations in tissue in this study are not above levels known to cause effects and thus the amphibians we tested in the SHP should not be at risk of contamination from the metals analyzed.

5. Conclusions

Amphibians inhabiting playas in the SHP are exposed to low concentrations of most metals. Sediment concentrations of metals were not different between land uses with the exception of Ba in grassland playas in grassland-dominated areas. It appears that metals are ubiquitously distributed and not concentrated in cropland playas. Concentrations of metals in amphibian tissues were lower than those found to cause effects in other studies and probably do not represent significantly toxic levels. However, toxicity was not assessed in this study; thus, no definitive conclusions about effects resulting from the metal levels observed in tissues in this study can be made. Nickel and Cd concentrations in *B. cognatus* differed between land uses, however no differences were observed for metal concentrations in *Spea* spp. In addition, *B. cognatus* tissue concentrations of Ba differed from those in *Spea* spp. in the same grassland playas but generally no differences were found in metal concentrations in tissues between species.

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